CLASSIFICATION OF THE ANAEROBIC BACTERIA[1]

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The classification of living forms should depend on an understanding of the laws of heredity as demonstrated in those forms. Preliminary classifications are made by applying the machinery of arrangement that has been worked out for other groups that are well understood, to groups of whose biological processes we know little. Preliminary classifications are necessary and are as desirable as are catalogues, and should be made to correspond to the known life processes of the organisms as nearly as possible, but they should only be offered tentatively.

The study of the biology of the anaerobic bacilli is in its early morning twilight. Today the scientific world holds two widely opposite opinions in regard to the classification of these organisms. The view held in Western Europe and in America is that the anaerobic species are many, distinguishable, and not highly variable; that held by many workers in Central Europe is that the species are practically indistinguishable, are highly variable, and may be changed one into another. It is impossible to bring these two points of view into alignment. They are not to be attributed to diverse interpretation of differences that shade into one another. They are themselves the outcome of an evolutionary process, depending on a mutation in thought, followed by the throwing up of a geographical barrier in 1914 that isolated the mutant thought and permitted it to overgrow its ancestral types of reasoning in a peculiarly favorable soil. The crux of the matter lies in the purity of the cultures studied by the classifier. It is notorious that the casual worker with anaerobic organisms knows neither how to purify them, nor how to tell when they are pure or impure. The anaerobes are not difficult to isolate when one knows how, but usually workers do not know how. Anaerobes have occasionally been isolated in pure culture since the early days of bacteriology.

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A number of descriptions of pure cultures exist, but it was the exceptionally studious worker who was responsible for such descriptions, and casual workers were apparently in the majority.

In 1901 GRASSBERGER and SCHATTENFROH (18, 19) propounded their theory of the transmutability of anaerobic species. In the period following they corroborated and extended their findings, and their work was pushed with so many publications (16, 17, 20–23) and with so much assertion that by 1914 it was seriously quoted in at least one well known German textbook (39), and the doctrine was thoroughly distributed throughout Central Europe. Under pressure of war, work is not carefully done. The casual workers found it necessary to make many anaerobic diagnoses from gas gangrene cases, which they made rapidly, and then turned to their new found collections to classify them. Many of these war workers corroborated the general findings of GRASSBERGER and SCHATTENFROH, namely, that the characters of anaerobes are highly variable, and that species among these organisms are not to be seriously studied.

CONRADI and BIELING (4, 5) were the most extreme in their contentions, claiming that one labile species was responsible for all gas gangrene cases. They described two cycles, one developing on carbohydrate media, the other on protein media, and claimed that immune sera identified all the strains in each cycle, but that when a strain was changed over to the other cycle culturally, it was also changed immunologically to that cycle. Such contentions struck so forcibly against all conceptions of immunity that they did not long go unchallenged. A number of workers (12, 38) corroborated the transmutability findings but not the immunological ones. In fact KLOSE (31, 32), working with highly variable impure cultures, used toxin formation to distinguish his strains. Some, such as ASCHOFF (1), who worked with slightly impure cultures, remained on the fence in regard to the transformations. Most assertive in their contentions that anaerobic organisms are highly variable in their reactions and are transmutable were the school of KOLLE (34–36), especially SCHLOSSBERGER (43), who suggested that the anaerobes may represent a single species. These workers sent cultures to VON WASSERMANN (49), who declared them indistinguishable. He

refused to submit anaerobic cultures to such drastic treatment as transatlantic shipment because of their fragility (48), and yet an anaerobic organism taken in muscle from a whale by NIELSEN (40) in 1888 responded satisfactorily to routine anaerobic technique in this laboratory in 1918, killing its guinea pig promptly and resembling exactly its original description! In the veterinary field VAN HEELSBERGEN (24) recently aligned himself with the German workers in human pathology.

The alteration of scientific attitude brought about by the adherence to such a theory as this is most interesting. Things become so simple under such an explanation, many technical difficulties are eliminated, immunization of animals for therapeutic purposes is made easy, and the scientific world, from the point of view of these workers, is so much the better off. For, if anaerobes may be changed one into another, why bother about isolating them? They will not stay pure. Anaerobic cultures from Central Europe that have found their way to this country are seldom pure, and frequently do not contain the type of organism for which they were named. If they contain a pathogen, two or three types may be isolated from the cultures, and these types behave consistently and do not do the queer things they were supposed to do by their first students. Central European anaerobic studies are struggling in the dark, the days of NÄGELI and BILLROTH have returned, the land of KOCH (33), of GHON and SACHS (15), and of VON HIBLER (30) has shifted from the careful work of older days, GHON himself (14) is converted to the new theories, and but a few constructive workers with abundant material have come out openly to combat them. Chief among these is ZEISSLER (51), a pupil of and coworker with FRAENKEL (6–11), who has always maintained that gas gangrene is due to various distinguishable anaerobic organisms. PFEIFFER and BESSAU (41) and GAEHTGENS (13) clearly distinguish various types. Early in the period of the war ZACHERL (50) and KÖVES (37) gave good descriptions of pure cultures of the vibrion septique type of organism.

As is to be expected, museum cultures from Central Europe are more badly mixed than any others. A collection of ten strains of anaerobes from KRAL'S in Vienna apparently did not contain a

single species for which the cultures were named. Unfortunately the anaerobe strains of some of our own institutions are but little better.

It would be difficult for the systematist employed in the study of higher plants whose major characters are well understood, whose mutations are today being scientifically studied, whose formal structure of classification was laid down many years ago and has been systematically developed, to imagine the complexity of the problem confronting one desirous of bringing order into the chaos represented not only by this war literature, but also by thirty years of anaerobic literature written before it. The time is more than ripe for some organization to enable new students to set to work with some clearness and assurance, an organization with a synopsis or index to the enormous literature that they must consult. This should give them an idea of the multiplicity of the species they will encounter, and should consider the biological factors relating to morphology, chemical behavior, and mutation as they are understood today.

Several workers have stated that anaerobic bacilli do not mutate. This is their natural reaction in denying the existence of the type of mutation that was described by the workers with impure cultures. To state that a living strain does not mutate would be to claim that it lacks one of the best recognized attributes of living matter. Obviously, it is necessary to determine where the mutations of bacteria lie, and what range of possible change they cover, before one can tell what characters are stable enough to be used for systematic purposes. The enzymes of the anaerobic bacilli are among the most highly active chemical agents known. Some of the anaerobes will be found among the most active splitters of carbohydrates, others have almost unbelievable proteolytic powers. It is to be expected that mutations will frequently be encountered in highly specialized organisms of this sort, and that these mutations will be chemical in their nature. When a mutation occurs that enables the organism to render assimilable a substance that its parent was unable to utilize, the mutant is readily detected because of the larger colony that it produces. Likewise a bacillus that loses a metabolic power forms smaller colonies than its parents. Data

pertaining to such mutations may be recorded photographically (28), and the possibilities thus afforded for the study of bacterial mutations in certain groups, notably in that of the tetanus bacillus (29), are unlimited.

The Society of American Bacteriologists (2) proposes the use of the botanical rules adopted at the Vienna Congress (44) in 1908 for the purposes of bacterial classification. In many ways the scheme formulated during a century and a half by the botanists is excellent for the purpose, although in some ways we are not ready for it. It is composed of stems and twigs and branches. When we pick up a bacterial group, we do not know whether to call it a stem or a twig or a branch, for the leaves have mostly grown on trunks. The tendency has been to work downward, to call a superficially recognized group a species and subdivide it into types, and to number the types. Why not work upward, call the numbered types species, and have more room for classification?

Bacteriologists, trained in pathological laboratories, have perhaps laid too little emphasis on the necessity of observing the laws of heredity in making classifications. It seems as though an application of these laws, with the same scale of nomenclature used by the classifiers of higher plants, might well be applied to the systematic arrangement of bacteria. Thus a tetanus strain of a pure biotype may give rise to many biotypes, as shown by colony formation. These derivative types are all typical tetanus bacilli. They represent elementary species, and are too many to catalogue, being of interest only to the student of heredity. They are no more deserving of specific names than are the commonly observed small mutations of higher plants and animals, and if named would require a trinomial nomenclature. There are some definite protein substances, however, differing radically in various tetanus bacilli, that probably are not subject to active mutation and are demonstrable by an immune reaction, agglutination. Four groups of tetanus bacilli have thus been distinguished by TULLOCH (45, 46), and four groups of vibrion septique bacilli by ROBERTSON (42). In the colon-typhoid group this reaction has long been considered specific, why not in these? To be sure, the details of the reaction

in these groups require more study, and other and better ways may be found to divide them. The following general rules will probably be found convenient for classifying bacteria.

BIOTYPE.—Strains that differ from each other in characters that are readily subject to mutation, and that breed true, may be termed biotypes. The word subspecies has so long been used in the higher groups with a geographical connotation that it will not be well to use it for subdivisions of bacterial species. The term type may then well be left as an independent unit of our vocabularies for non-specific use.

SPECIES.—Strains that behave alike in those characters that within their genus have not been found to mutate readily, may be grouped as species. The occasional derivation of one species from another is no more to be considered impossible than it is in higher groups. Bacteriologists have too long considered the species conception in higher groups as one of fixed immutable orms, which it is not. The recombination effects noted by DE VRIES (**47**), in which he showed the independent origin of three well defined types (*Oenothera nanella*, *O. eliptica*, and *O. lata*) from two others of quite widely divergent character (*O. Lamarckiana* and *O. laevifolia*) apparently cannot occur among bacteria. These recombination effects allowed the sudden appearance of groups of mutations that had occurred previously. Among bacteria, however, because of the rapidity with which vast numbers may be bred and the energy with which selection acts, several characters may be changed nearly simultaneously, and similar effects to those noted by DE VRIES may occasionally be observed, namely, the appearance of a number of new characters within a short space of time. On analogy, it would be perfectly reasonable to describe the strains that result from various changes as separate species. It is quibbling to define the word "species" so closely that no elasticity should be allowed in its application. Our knowledge is too meager and the possibilities too great to restrict closely the meanings of taxonomic words. Changes that may be considered specific may be discovered or perhaps even brought about by treatment of bacteria that is more radical than anything tried with plants, and there is no reason why

a bacteriologist who finds a new type that has originated in his own hands should not dignify it by the name of species, if he can show that such a change affects several characters.

GENUS.—Organisms that show the same general reactions on ordinary media and have the same general morphological habit may be grouped in genera. Such a scheme will compel the classification of most of the old and well recognized anaerobic species as genera, although it will unite some, such as NOVY's bacillus and *B. oedematiens*, and vibrion septique and the whale septicaemia bacillus into genera. It gives to the words species and genus approximately the same rank, in relation to mutational possibilities, as they possess among the higher forms. A vibrion septique strain can no more mutate to a sporogenes habit than can a pine tree mutate to an oak, but it can mutate in small detailed characters that may be of interest, and there are vibrion septique strains that differ in more fixed characters and that should be given specific differentiation.

A detailed plan (**27**) following these lines has already been presented. It is intended as a preliminary classification. Twenty-seven anaerobic genera have been defined, many more will have to be admitted in time, especially in the proteolytic group, and when large collections have been studied many emendations will have to be made. An organization (**26**) of these genera has been prepared, employing chemical criteria only; morphological criteria are found entirely inadequate for purposes of classification in this group. It has been thought premature to group the genera into tribes. Two subfamilies include all the anaerobic rods described in the genera.

Subfamily I. Clostridioideae.— Clostridiaceae that on meat medium produce after twenty days' incubation under oil at 37° a reaction of P_H 7.0 or a more acid reaction, the culture having been boiled after incubation. Type genus *Rivoltillus*, the vibrion septique type as described by HELLER (**25**).

Subfamily II. Putrificoideae.—Clostridiaceae that on meat medium produce after twenty days' incubation at 37° under oil a reaction of P_H 7.1 or a more alkaline reaction, the culture having been boiled after incubation. Type genus *Metchnikovillus*, the sporogenes type as defined in the description of *Bacillus sporogenes*,

described by the Medical Research Committee (3) under the name of Metchnikoff's type A. These subfamilies are united into the following family:

Clostridiaceae.—Eubacteriales that are rodlike, not spiral, that will not grow within 7 mm. of the surface of a shaft of clear tissue-free agar medium contained in a tube 12 mm. or more in diameter, incubated in air, in which they are able to grow in the depths. They may or may not possess peritrichial flagella; they may or may not form endospores. Most members of the group are characterized by their energetic catalytic action on proteins or on carbohydrates or on both of these types of substances.

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